The Development of Symbranchus marmoratus.

By

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With Plates 1—4 and 4 Text-figs.

Contents.

I. Introduction.

The Symbranchii are a small group of eel-like fishes which are generally placed near the Apodes in recent systems of classification, though lack of sufficient anatomical description and embryological material has made it difficult to determine exactly their affinities. Tate Regan, in his "Anatomy and Classification of the Symbranchoid Eels" (**9**), derives them from the Acanthopterous physoclists. The embryology of a Symbranchid has, up till now, not been investigated.

The following account deals with the South American species, Symbranchus marmoratus, which long ago excited attention because, while possessing well-developed gill-lamellæ on each of the four gill-arches, and typical Teleostean aortic arches, it yet spent a considerable part of the year buried in the mud like a Lepidosiren (**5**). Probably correlated with this habit of burrowing, and with modifications in the respiratory processes during the dry season, is the single median ventral branchial opening—a character which is described in the name Symbranchus. Other adaptations to this burrowing habit are probably the great length of the gill-chamber—the branchial opening being situated comparatively far back—the vascular character of its lining, and also its almost complete separation from the exterior. Several members of this group possess accessory respiratory sacs, and J. Müller, in his work on the Myxinoids, says: "Es wird wohl das obere Ende des Zungenbeins gemeint sein, denn hier sehe ich bei Symbranchus eine blinde Vertiefung jedoch ohne Sack, zwischen dem Zungenbein und dem obern Ende des ersten Kiemenbogens."

I have found the diverticulum referred to by Müller in the three adult specimens which I have examined—and indication of its future position in the older larvæ. An account of its structure will be given in the section on the alimentary canal.

The material for this work was obtained during an expedition to the Gran Chaco in 1907, when Dr. Agar made the interesting discovery that the larva of Symbranchus has very well developed pectoral fins. Hitherto the absence of pectoral fins has been regarded as one of the characteristics of the group. The material consists of eggs and larvæ preserved immediately after they were taken from the nest, and also of specimens reared under artificial conditions in tanks and dishes of water. In the latter case it was possible to record the rate of growth after hatching.

Dr. Agar has given an account of his expedition in his paper on "Spermatogenesis of Lepidosiren paradoxa" (**1**), and to his field notes I am indebted for all the details relating to the nest of Symbranchus, the collection of the eggs, the habits of the living creature, while what I say regarding "movement of the fins" is taken verbatim from his notes. I should like to take this opportunity of thanking Dr. Agar for placing this unique material unreservedly at my disposal, and for the kind interest he has shown in the progress of the research.

My best thanks are offered to Professor Graham Kerr, in whose laboratory much of the work was done, and who has helped me throughout with constant advice and criticism.

I am greatly indebted to Sr. Veronica, S.N.D., for her artistic illustrations.

Symbranchus, like many fishes and amphibians, exhibits the phenomenon of colour-change in response to alteration in the conditions of light and darkness, specimens fixed at night being lighter in colour, because of the contraction of the chromatophores, than those fixed in daylight. The living larvæ actively burrow among the weeds at the bottom of the rearing tanks when the lid is removed—as though to escape from the light.

About a dozen nests in all were found, the adult male, as in the case of Lepidosiren, being present to guard the contents. In many other respects the nesting habits of Symbranchus resemble those of Lepidosiren.

The water in the swamps where the eggs were found was commonly from one to four feet deep. The mouth of the nest opens on the bottom of the swamp and leads into a tunnel running obliquely downwards into the mud. At the end the tunnel takes a more or less horizontal direction, and it is in this part that the eggs are laid, there being little or no weed.

The eggs are translucent, and of a greyish colour. It is quite impossible to assign a definite age to the contents of any one nest, as the eggs may show every variety in state of development from "early segmentation" to "moving embryos." Neither is it possible to give any definite idea of the rate of development before hatching. However, in a nest discovered on December 12th the eggs were of fairly uniform and of early stage (Stage 5). Some of these hatched out on the 19th, the resulting larvæ being of the stage described as

24. Some of these same eggs, extracted and preserved on the 17th, are described as Stage 23.

For the following descriptions, whole and sectioned eggs and embryos fixed in corrosive acetic and formalin were examined, as well as stained and cleared preparations of blastoderms and of whole embryos removed from the underlying yolk mass. The external appearance of the embryo varies a little with the fixative employed, as may be seen by comparing Pl. 1, figs. 5 and 5 A. These two eggs are of about the same age, but that figured in 5 A was fixed in corrosive acetic, that figured in 5 in formalin.

For stages beyond 24 whole specimens stained and cleared in xylol were consulted as well as opaque specimens and sections.

II. General Sketch of Development.

The preserved specimens up to Stage 20 were mostly collected from three nests, and were fixed in formalin, warmed or cold. No eggs were found in the cœlom of female Symbranchus.

The laid egg is spherical and has a diameter of 3·4 to 3·5 mm. Its capsule is firm and somewhat tough and separated from it by fluid. A mass of what looks like a coagulated albuminous substance is present at the vegetative pole. When the capsule is removed this substance separates away, so that the egg appears to be sometimes no longer spherical but somewhat oblate, the equatorial diameter being the greater. It is also very brittle.

The youngest specimen in the collection is described as Stage 5 (Pl. 1, fig. 1). At this stage the blastoderm is very well marked. It looks like a white circular pad "let into" the yolk-mass, this pad being composed of small white cells and measuring about 1 mm. in diameter. It stands above the surrounding yolk to a height of nearly ·5 mm. The animal pole is flat in comparison with the distinctly spherically curved vegetative pole.

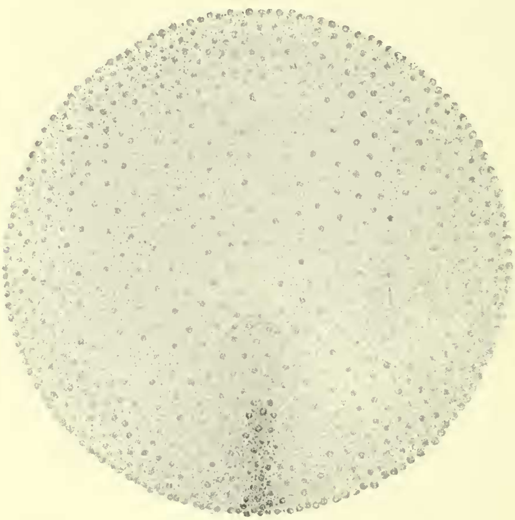
Celloidin sections through eggs of this stage show that this pad is more or less bi-convex in shape—9 to 10 cells deep in the centre. Already an outer layer of cells is becoming marked off from the general mass, i. e. an ectoderm differentiating. Three distinct zones can be seen in the subjacent yolk, from which the pad can easily be separated. Under the central deepest part of the blastoderm the yolk is finely divided, while the lateral parts of the "pad" overlie a mass of protoplasm studded with large nuclei. Here the outer rim of the blastoderm shows more obvious continuity with the rest of the egg than it does over the central portions. This protoplasmic mass no doubt functions in connection with the passing on of food material to the quickly dividing cells in the blastodermic area.

Protoplasmic masses and disintegrated yolk pass more or less suddenly into the large structureless yolk-blocks which constitute the vegetative part of the egg. A thin film of protoplasm acts as a covering to the yolk-mass, which is not yet covered by blastoderm. This covering is, however, a very slender protection, especially at the vegetative pole, where masses of yolk break away very readily when once the capsule has been removed.

Stage 7.—As the blastoderm grows the central portion appears to thin out, while the rim is thickened and less white in appearance. The yolk nearest the blastoderm is much lighter in colour than the rest, which is brownish-yellow—this difference in colour being the external expression of the yolk differentiations described above. The celloidin sections show an ectoderm, now two-layered, and an irregular and very small segmentation cavity.

Stage 10.—When the blastoderm has covered up about 100° to 120° the thickening of the rim becomes accentuated at one point to form the embryonic rudiment (Pl. 1, fig. 2, and Text-fig. 1). In the paraffin sections (Pl. 3, fig. 17) there is a space underlying the embryo rudiment which may be artificial or which may represent remnants of an archenteric cavity. Celloidin sections (Pl. 3, fig. 18) do not show any such space, but the series is a slightly older one than the paraffin series. In the embryonic rudiment, as seen in a celloidin section, the thickened neural plate covered by ectoderm lies over an ill-defined endoderm, the yolk syncytium separating the latter from the yolk. The rest of the blastoderm is now two cells deep except at its advancing edge, where it is still thick.

Text-fig. 1.



A microscopic preparation of the blastoderm of an egg of Stage 10, removed from the underlying yolk, stained, cleared and mounted in balsam.

Stage 11 is illustrated by Plate 1, fig. 3. The blastoderm is gradually extending over the egg, becoming much thinner as it does so, as though the increase in surface area were at the expense of depth. The embryonic rudiment is elongated to form a white streak, which is broad at the anterior end, narrower in the middle, and broader again where it gradually fades into the blastodermic rim. The elements forming this streak appear, in sections, to be very much pressed down by the outer ectoderm.

The small size of the egg and the corresponding small size and flattening of the embryonic rudiment make it practically impossible at this stage to do more than distinguish the brain rudiment from that of the neural tube and myotomes. A faint median longitudinal groove can be seen externally. A microscopic examination of sections of the egg shows that the broad anterior part of the rudiment consists of a wide-spreading, solid medullary plate overlying a one-cell-deep plate of endoderm, there being little mesoderm. In the middle parts of the rudiment a solid keel—the future spinal cord—is quite easily distinguishable from the mesoderm and from the endoderm. There is no certain trace of notochord yet.

The groove alluded to above is the external expression of a very shallow mid-dorsal depression found in the anterior parts of the embryonic rudiment, which disappears when the flat, wide-spreading medullary plate is converted into the solid uprising structure which forms the central nervous system just before the cavities are hollowed out.

Stage 17.—When the still uncovered circular yolk-patch measures about 48° the embryo rudiment is about 1·8 to 2 mm. in length (Pl. 1, fig. 4), though there are great variations, length being not always proportional either to differentiation of embryo or to size of uncovered yolk-patch. By this time the front part of the medullary plate has begun to rise from the egg, and to form the solid brain, and this uprising gradually extends backwards to the rest of the neural rudiment. The myotome rudiments are not yet differentiated into dorsal and ventral portions. The notochord is present, but its derivation is difficult to observe. The solid rudiments of nose, eyes and ears are not yet distinct, though in the next stage—when the lumen of the nervous system appears—they are easily distinguishable in sections and their ectodermal connections can also be seen. Externally, however, these organs are not yet visible.

As development proceeds, liquefied yolk or oil, of which there is much in the yolk, seems to concentrate in front of the embryo, and then to spread out somewhat so as eventually to extend over about half the sphere. This gives the egg a very characteristic appearance, the liquid yolk being silvery and glistening.

Stage 19.—By the time the embryo is 2 mm. or a little more in length the yolk has been quite covered in. The brain is hollow. The rudiments of eyes and ears are assuming a cavity; the position of the principal cranial nerves can be determined, as well as that of mandibular and hyoid arches and the rudiments of the four branchial arches. A most careful search, however, has revealed no trace on the external surface of gill-slits, either in this or in later stages, the slits not becoming perforate till after they are covered over by the operculum, so that in this respect Symbranchus differs from other Teleosteans. The developing pectoral fins are conspicuous in sections at this stage. They contain the solid ventral muscle processes of three myotomes of the front part of the trunk. The innervation and muscularisation of the pectoral fins has been worked out by a study of the composition of the brachial plexus, an account of which will be given later on.

No trace of Kupffer's vesicle can be found in this or in earlier stages.

Stage 21.—The embryonic rudiment has now a ridge-like appearance. It curves round the yolk-sac, which has a diameter of 2·9 to 3 mm., through an angle of 170°. By the aid of sections the brain is seen to be divided into three parts, though the mesencephalon is not nearly so well marked off from the prosencephalon, as it is from the rhombencephalon. The optic vesicles are conspicuous, while the mandibular, hyoid and branchial arches with their nerve supply are to be seen as an unsegmented, translucent, tripartite mass lying on either side of the brain (Pl. 1, fig. 5). The fins look like two patches on the surface of the yolk, the ventral processes of the myotomes (Pl. 1, fig. 5A) like oblong blocks spread out on the dorsal part of the yolk-sac. The pericardial cavity is beginning to form. Pl. 1, fig. 5 gives a view of an egg slightly older than the one just referred to. The main mass of the pectoral fins is about ·3 mm. from the embryo proper, so that the fins look like two white mammillæ apparently unconnected with it (see also Pl. 1, fig. 6, a slightly older stage).

Stage 23.—When the embryo is a little over five days old there is a curious proboscis-like structure (see Pl. 1, fig. 7), projecting freely from the extreme end of the creature more or less on a line with the mesencephalon. This rostrum is transparent and bluntly pointed and filled apparently with a clear fluid. The tail is curled, though still in contact with the yolk. The pectoral fins are about ·5 mm. in breadth and extend upwards and backwards over the yolk. The cartilaginous skeleton of the fin has made its appearance and is visible externally.

Stage 24 (Pl. 1, fig. 8) is the stage at which the embryo probably hatches out. The body of the creature has become much more cut off from the yolk, the rostrum projecting forwards anteriorly, the hind end being quite free. The heart is visible in the clear space separating the head from the yolk mass. The visceral arches are more conspicuous; eyes and nostrils are ventral in position. Two rounded optic lobes anteriorly and a well-marked very wide rhombencephalon posteriorly seem to be covered with a transparent lymph space continued into the rostrum already alluded to. The unpaired fin is beginning to grow.

Stage 25 (slightly more advanced than Pl. 1, fig. 8).—The embryo has now considerably lengthened, the posterior part being coiled as a result of this growth. As in the last stage, the head does not project freely from the yolk-sac except the rostrum, which is now extremely conspicuous, being about ·7 mm. long. In some of the specimens particles of mud and débris are attached to the rostrum at its tip, which suggested that this structure might perhaps function as a cement organ. A microscopic investigation of such specimens has, however, given little, if any, support to such an interpretation of the function of this curious appendage.

The organ seems to be a prolongation of the transparent tissues surrounding the dorsal part of the brain, filled with a lymph-like fluid without any apparent cellular elements and walled in by two layers of epidermis, the outer of which is flattened and horny (Pl. 1, fig. 11). In the specimens with the débris there is a slight thickening of the lower epidermis cells in the neighbourhood of the débris which may possibly represent a vestigial cement-gland, but it has not been found possible to demonstrate a break in the continuity of the outer horny layer, which fact tells against such an interpretation of the organ. The line of demarcation between the mesenchyme of the head and the fluid contents of the rostrum is very definite.

There is a resemblance between the figures of the early developmental stages of the rostrum of Callorhynchus (**10**) and that of Symbranchus, and also a certain amount of resemblance between their structure in the early stages, for Schauinsland says that the rudiment of the rostrum of Callorhynchus is a large thin-walled sac covered with ectoderm and later becoming filled with mesoderm—a description which might apply in part to the organ in Symbranchus.

Large unicellular glands are very conspicuous objects in the ectoderm in sections stained in Heidenhain and eosin. They are most abundant in the epidermis of the fins, but occur also in the general epidermis, and are to be found, though sparsely, on the rostrum. These glands are evidently larval structures, for they gradually disappear as the adult stage is reached.

By this stage the dorsal and ventral median fins are well established and there is a cloacal opening which appeared in Stage 24, so that it is now possible to distinguish the anterior limit of the true tail. In dorsal view the only parts of the brain visible are the optic lobes and rhombencephalon because of the cranial flexure. In side view the infundibulum and hypophysis are conspicuous; olfactory organs and eyes have become more lateral in position. The heart is more clearly seen, the ear is still visible (in the xylol specimens) anterior and dorsal to the branchial regions. An operculum barely covering the first branchial arch is visible in horizontal sections. The pectoral fin, the anterior edge of which curves backwards, now measures about 1·1 mm. in length. The liver is visible on the left side in the xylol specimens.

Dr. Agar describes in his notes a pigment-spot on the left side of the creature as being conspicuous in the fresh specimens. In the early stages, i.e. forty hours after hatching, this pigment-spot lies anterior to the middle point of the attachment of the yolk sac. Its position is relatively further back in the later stages of development and more superficial. No trace of this pigment-spot can be found in the preserved specimens, but in those stained and cleared in xylol the gall-bladder is distinctly visible in the position described in the notes, and a consultation of the sections leaves little doubt that the gall-bladder is responsible for the spot.

Stage 26 (Pl. 1, fig. 9).—The coiled portion of the body alluded to above straightens out as the creature grows, so that at this stage (26) a straight line joining the tip of the rostrum to the tip of the tail measures 7 mm. The rostrum has lengthened and lies parallel with the curve of the yolk-sac. The pectoral fins are about 1·5 mm. in length. The cerebellum rudiment is conspicuous (*c. r.*, Pl. 1, fig. 9); the eye is more lateral in position. The operculum covers the first two branchial arches.

Stage 27.—Seventy hours after hatching the creature is about 9 mm. long, the true tail being about 2·5 mm. Its general appearance has, however, undergone little change from the conditions shown in Pl. 1, fig. 9. The pericardial space has increased in depth. The head is becoming more opaque.

In Stage 28, the rostrum, on account of the growth of the brain, nose and eyes would now be described more correctly as a dorsal cephalic process than as an anterior one. The subintestinal vein (Pl. 1, fig. 9) is conspicuous in the figures of this and succeeding stages, and a sketch of the living creature among Dr. Agar's field notes shows that it is evidently a striking object in the living larva. The sections show that the blood-vessels of the fins are well established, the arterial supply being derived from the dorsal aorta in the neighbourhood of the pronephros immediately behind the heart, the venous blood being returned to the duct of Cuvier.

In Symbranchus it is impossible to say dogmatically that the artery to the pectoral fin has been derived from an intersegmental artery (i. e. is a primary subclavian artery[1]) because of the precocious development of the fin in question, but a comparison of the early development of the fin artery with the intersegmental arteries (Pl. 4, fig. 27) that arise later on when the myotomes are fully formed shows that the arterial supply to the fins is intersegmental in nature. Three myotomes take part in the formation of the fin; whether the subclavian in Symbranchus represents the intersegmental artery of one or the fused intersegmental arteries of all three myotomes it is not possible to say.

Stage 29.—There is a shrinking in the rostrum at this stage. From now onwards this structure gradually diminishes in size. Chromatophores appear, being confined to the head at first. On the right-hand side of the creature the cœlom is visible externally, the liver being conspicuous on the left.

In Stage 29 the inclusion of the yolk in the abdominal cavity commences. This has an important effect on the contour of the creature, as may be seen by comparing Pl. 1, fig. 9, and Pl. 2, fig. 13.

Stage 30,[2] (Pl. 1, fig. 10).—The creature at this stage is 13 or 14 mm. in length, its rostrum being 1 mm. long. There has been a great forward growth of head, which is assuming a definitive form, the optic lobes are covered with a pigmented epithelium, cranial flexure has almost disappeared. The eyes are fully developed, and in contrast to later stages are only just visible in dorsal view.

[1] Hochstetter in Hertwig, O., 'Handbuch der vergleichenden und experimentellen Entwickelungslehre der Wirbeltiere.'

[2] In writing the description of this stage I have been aided by a large drawing made by Dr. Agar from the living larva.

The living larva makes frequent swallowing movements and the gill-lamellæ are differentiating. Thus preparations for branchial respiration are afoot. Hitherto respiration has been effected mainly by the pectoral fins. The larva has also begun to feed, its food under artificial conditions consisting of earthworms and liver. In the living specimen dorsal aorta, posterior cardinal, caudal, subintestinal veins are well marked, while the ventral aorta and the four aortic arches can be seen. The urinary bladder, the widened out posterior parts of the fused archinephric ducts, first visible in Stage 28, is conspicuous in the xylol specimens of this stage. It now measures 1 mm. in length and lies dorsal to the rectum, opening to the exterior immediately posterior to the anus, the cloaca having flattened out.

Description of the Pectoral Fins.—It has been shown that in the early stages, 18 to 21, the pectoral fins consist of mesoderm masses—the ventral processes of certain anterior myotomes—covered by a two-layered epidermis. Very quickly, however, these masses begin to differentiate. Cartilage derived from the cells forming the central part of the fin-rudiment appears at Stage 24 to form the skeleton, which is visible externally, and the muscle-fibres are differentiated. The main mass of the fins, however, is mesenchymatous, the increase in size being mainly due to increase of this tissue. The fins are very transparent and beautiful objects as well as conspicuous by their large size, the longest diameter at Stage 30 being 2·5 mm. When living they are quite red because of the rich network of capillaries. They are roughly semicircular in shape, the diameter of the circle lying nearest to the yolk-sac. The cartilaginous part of the fin is triangular (Pl. 1, fig. 10 and Pl. 2, fig 12), the apex of the triangle consisting of a rounded knob, to which most of the muscles are attached. This rounded knob fits into the shoulder girdle, dividing the latter into a dorso-lateral and ventro-lateral portion. The shoulder-girdle is little more than a thin lamella of bone surrounded by much muscle. The cartilage in the fin is differentiated earlier than the bone of the shoulder girdle. I have not observed any trace of cartilage in the development of the girdle.

The unicellular glands already described are visible externally under a lens.

Three main blood-vessels are distinctly visible in the fin, one afferent supplied from the dorsal aorta which runs into the fin dorsal to the "skeleton," and which breaks up into capillaries, and two efferent. These efferent vessels make their appearance in the circumferential regions of the fin. One of them then takes a route dorsal to the "skeleton," the other ventral. Both pass into the body proper anterior to the nodular end of the cartilaginous skeleton and to the shoulder girdle, and having joined, they pass backwards parallel to the inferior jugulars to open into the heart at a little distance behind the junction of the duct of Cuvier with the heart. This backward course is due to the fact that the position of the heart has been changed relative to the fins since the veins were first formed.

The innervation and muscularisation of the fin has been worked out by reconstructions made by the Graham Kerr method (**4**) from sagittal sections. In the earlier stages transverse sections are useful. The occipital arch was taken as a fixed point, myotomes and nerves behind this arch being numbered 1, 2, 3, etc. It can be shown that spinal nerves 1, 2 and 3 innervate the fin. This is an indirect method of proving that it was the ventral process of the first three trunk myotomes that were concerned in the formation of the fin, but as late as Stage 26 there is no brachial plexus—each nerve enters the fin quite independent.

In Stage 28 the ventral ganglia of the first and second spinal nerves, which, unlike the other spinal nerves, were not separated by neural arches, are fusing one with the other. This fusion, which is probably correlated with the lengthening-out process of the branchial cavity already alluded to, has gone still further in Stage 29, though the double character of the apparently first spinal nerve is still evident. The brachial plexus begins to differentiate in Stage 28, the plexus being somewhat diffuse and wide spreading at first.

A reconstruction of Stage 30 shows that the fused spinal ganglia of nerves 1 and 2 forms a large structure just outside the occipital arch. It also shows that the shoulder girdle has suffered a backward migration relative to the spinal nerves, for nerves 1 and 2, now fused into one nerve, and nerve 3, to a less extent, have to run backwards more or less parallel to the vertebral column to join the brachial plexus. This backward displacement of the shoulder girdle has resulted in Stage 33 in the further elongation of the nerves running to the brachial plexus. A small branch of spinal nerve 4 joins the plexus, though I have no definite developmental evidence that myotome 4 has played any part in muscularising the fin.

Movements of the Fins.—As soon as they are hatched the larvæ begin to make constant movements with their great vascular pectoral fins, doubtless for respiratory purposes; the motion is jerky and very characteristic. Each fin is moved through an angle of about 180°, being flat against the body at the end both of its forward and backward movement, and the direction is generally opposite on the two sides, one fin being flapped forward while the other is being turned back. This produces an impression on the observer watching the larvæ from above as if the two fins were a single bar pivoted through its centre to the body of the larvæ and surging backwards and forwards in a jerky manner. This form of motion is not absolutely fixed, however, and when the larvæ were disturbed they were sometimes seen to flex and extend their fins together, thus using them as ordinary locomotor organs.

The further history of the pectoral fins is given under Stages 32, etc. No trace of pelvic fins has yet been forthcoming though a careful search has been made.

A fuller history of the gill-chamber and the opening is given in connection with the development of the alimentary canal; here these structures will be only briefly described. Gill-slits such as are seen, for example, in a developing trout are never visible on the exterior of Symbranchus. The branchial chamber, which is gradually hollowed out as development proceeds, develops a connection with the exterior by means of a small pore on each side, microscopic in character, dorso-lateral in position. These openings lie behind the posterior edge of the operculum and are found in the early stages (up to Stage 29), when the pericardium is still ventral to the pharynx. At Stage 30 the heart has suffered a backward migration relative to the gill-chamber, and by this time an additional opening to the exterior from the latter has been burrowed out on the ventral side of the creature. This ventral opening becomes continuous with the dorso-lateral pores on each side, thus forming a single crescent-shaped opercular opening, the horns of the crescent being posterior and dorso-lateral in position. If the ventral part of this opening could be obliterated then the opercular conditions of Symbranchus would resemble those of an ordinary Teleostean, i.e. there would be two lateral openings. However, it will be shown that, as development proceeds, the operculum continues to grow backwards, and as it does so it fuses with the body-wall, so that eventually a single median ventral branchial opening is the result. Co-extensive with this backward growth of the operculum is the lengthening of the branchial arches and of the whole gill-chamber.

Stage 32 (Pl. 2, fig. 13).—About eight days after hatching the creature begins to assume something of its adult appearance and to use its gills quite freely. It is about 18 mm. long, having a tail of 5 mm. in length. The yolk-sac has almost lost its globular appearance, and the inclusion of the yolk in the abdominal cavity, already alluded to, has continued. The yolk enclosed in the body of the creature now extends to within 1 mm. of the anus. The body anterior to the heart is much elongated, the most anterior point being at a distance of 2·5 mm. from the yolk-mass. The pectoral fins have, however, not grown in proportion to the rest of the animal: they are about the same actual size as in Stage 30. Hence, on account of the general growth of the creature, they are not so strikingly conspicuous as in the younger stages.

The increase of distance between the anterior point and yolk-mass is due not only to the lengthening of the gill-chamber, but also to the formation of a snout, which is now quite clearly defined, and which has grown out from under the cerebral part of the brain. The telencephalon is in consequence now nearly at the same level as the optic lobes. This snout formation has the effect of carrying the eyes to a more anterior position: they now lie under the cerebral regions quite anterior to the optic lobes. The plane of the mouth opening becomes rotated upwards so as to assume a definitive horizontal position.

The rostrum has almost disappeared. Chromatophores are increasing in number; the creature is more opaque, so that liver and bladder are less conspicuous in the xylol specimens. The median dorsal fin in Stage 32 is dying away anteriorly. It begins to rise from the general surface behind the gill-chamber.

Stage 33 (Pl. 2, fig. 15).—Thirteen days after hatching the creature is about 21 mm. long and the yolk-sac has completely disappeared. A little mound—striking, because of the absence of pigment, which by now is abundant all round it—is all that remains of the rostrum. The root of the pectoral fins has become shifted ventrally nearly to the mid-ventral line. The lateral parts of the crescentic opercular opening of earlier stages are gradually being closed up, so that in this stage the opercular opening is quite ventral though relatively larger than in the fully adult condition.

The posterior parts of the outer wall of the gill-chamber, especially in the neighbourhood of the opening, are exceedingly thin. Normally the exit from the gill-chamber is closed and concealed, except when water is being actually expelled from it. The shoulder girdle and the attachment of the fins are now quite anterior in position to the hind end of the branchial chamber, the opercular flaps overlapping the base of the fins, which are now somewhat crumpled instead of being flat. It will be remembered that in Stage 30 branchial respiration was just beginning, and that the pectoral fins ceased growing as soon as branchial respiration was well established. In Stage 34 the fins shrivel and fall off bodily, the discarded fins measuring about 1·5 mm. in length. The "stumps" are, however, recognisable in the sections for some time longer. Quantities of these discarded fins are to be found lying on the bottom of the tanks containing living larvæ of this and the succeeding stages. The fins in Stage 30 are situated just posterior to the opercular opening. In Stage 32, however, the hind end of the operculum has reached the point of origin of the fins, as may be seen in Pl. 3, fig. 19. In Stage 33 operculum and branchial cavity have become elongated to such an extent that the fin "stalks" are actually covered by the operculum, and can only gain exit from the branchial chamber on the ventral side (Pl. 2, figs. 14 and 15), the gill-chamber opening by this time having nearly assumed its adult position and size.

The pressure of the edge of the operculum on the base of the fin is possibly responsible for the degeneration of the tissues in the "stalk"—thus enabling the main part of the fin to drop off. On the other hand, it is quite possible that the "shedding of the fins" is a natural selection phenomenon, the fins tending to degenerate at their base at the period when they become covered by the operculum without there being any direct mechanical effect. The next stage (34, which is not figured) is marked by absence of fins, a more anterior position of the eyes, and by the extension of the liver to the level of the anus.

In Stage 35 (Pl. 2, fig. 16) the larva has practically assumed its adult form, though head and branchial regions are perhaps more distinctly distinguishable from the rest of the body than they are in the full grown specimen. There is no sign of pectoral fins and no trace of rostrum. The creature is eel-like in shape, the body behind the opercular cavity being almost perfectly cylindrical, while the hinder part of the trunk, becoming gradually more laterally compressed, merges eventually into the tail. The ventral ends of the myotomes have now met ventrally and form a complete investment of muscles to the trunk. Chromatophores are still confined to the dorsal and lateral parts, the ventral surface being quite colourless.

The length is 26 mm. The true tail is distinguished by the continuous median fin, which gradually rises from the dorsal surface in front of the anus, is continued round the tip of the tail, and dies away on the ventral side at a point midway between the extreme hind end and the anus.

Although this continuous fin is only conspicuous in the tail, there is, however, a low-lying dorsal fin-like structure extending as far forwards as the pericardium. A similar structure continues forwards—the ventral portion of the fin—this being the last remains of the transparent tissues traversed by the sub-intestinal vein of the earlier stages.

The tail fin is still transparent, though branching chromatophores are now more copiously present. The anus is about 9 mm. from the tip of the tail.

The extreme front end of the snout is squarish, the upper jaw longer than the lower, so that the mouth is slightly ventral. The gape is not great, and is well protected by the thick fleshy upper and lower lips. Just inside the mouth-cavity on its dorsal surface there are some forwardly directed blind pockets, the floors of which form valvular flaps for the more efficient closing of the mouth aperture. Both anterior and posterior nares are visible, the latter, which first appear in Stage 30, being situated just in front of the eyes, the former at the very tip of the snout on either side of the middle line. The posterior nares do not arise by a division into two of one single olfactory opening, but by a hollowing out of a channel to the exterior.

Sensory canals, which first make their appearance in Stage 34 just posterior to the olfactory organ, are present in the head, opening to the exterior at intervals of about ·08 mm. The epithelium of the snout is richly glandular, as, indeed, is the epithelium of the trunk.

The opercular cavity stretches backwards to a distance of 4 mm., the opercular opening being now quite the small median ventral aperture so typical of the adult.

The ventral aorta is very long, the distance between the heart and the fourth aortic arch being 1·6 mm. In the full-grown specimen the distance between the tip of the snout and the pericardium is roughly a quarter of the whole length, and as a rule the pericardium bulges out slightly and is consequently visible exteriorly. The eyes are smaller in proportion than they were in the earlier stages.

III. Notes on the Development of Certain Systems of Organs.

A. Alimentary Canal and Certain of its Glands.

As has been shown in the preceding account, gastrulation occurs at about Stage 8. Celloidin sections through the egg of Symbranchus at Stage 10 and onwards show that yolk endoderm (yolk syncytium) underlies the whole of the blastoderm. In the embryonic rudiment (Stage 10) endoderm cells can be clearly distinguished from the overlying thickened neural plate (Pl. 3, fig 18, *n.p.*). The nuclei of this endoderm, which does not extend beyond the limits of the rudiment, in contradistinction to the large nuclei of the yolk endoderm, are small and oval-shaped, the long axis of each being usually at right angles to the axis of the embryonic rudiment. The line of demarcation between the endoderm of the embryonic rudiment and the yolk-syncytium is well defined in both paraffin and celloidin sections.

As the development proceeds, mesoderm gradually separates apart the endoderm and the overlying neural rudiment, and the notochord is formed. I have not been able to observe the process by which the notochord is derived from the endoderm, but by Stage 16 this structure is easily recognisable as a solid cord of cells.

In Stage 18 the stomodæum begins to grow in from the deep layer of ectoderm. It is very difficult to fix precisely its internal limits, but the balance of evidence from many sections seems to show that it does not extend inwards beyond the infundibulum and the hypophysis. The outermost horny layer is not involved in the growth in any way. The stomodæum is, of course, quite solid. The flat, laterally spreading endoderm of the preceding stages is becoming a more or less rounded, solid mass of cells in the stomach rudiment, which is very closely apposed to the overlying notochord.

The pharyngeal rudiment overlies the pericardium in Stage 19. The solid endodermal cleft rudiments are not yet clearly distinguishable.

Transverse sections of embryos of Stage 21 show that the anterior parts of the alimentary canal, including the visceral clefts, are gradually moulded during the course of development out of a solid mass of tissue, swollen out laterally and tapering towards the central axis, which begins posterior to the infundibulum. The position of the solid hyobranchial cleft rudiment can now (Stage 21) be defined, while behind the branchial arches the rudiment of the œsophagus can be distinguished as a solid mass, broader than deep. Posteriorly, a solid cord of endoderm cells, the gut-rudiment, stretches with little change, save a gradually decreasing diameter to the hind end. The process of separation of this definitive endoderm from the syncytial yolk-endoderm has not been observed.

The alimentary canal is still solid at Stage 24, the cloacal opening being established by Stage 25. The lining of the stomodæum at Stage 24 is differentiating into a very regular epithelium. Mandibular and hyoid arches can clearly be distinguished in sagittal sections, while in the branchial rudiment, although the clefts have not yet been hollowed out, it is easy to see the number of arches, because of the epithelial lining of each.

Transverse sections at Stage 24 are particularly interesting because they show clearly an ingrowth of ectoderm in connection with each of the branchial cleft rudiments. The branchial rudiment is still solid as explained above. These ingrowths of ectoderm are quite masked externally by the outer horny layer, which is not involved in the ingrowth. If this deep-seated layer of ectoderm is a true ectoderm—and there seems no reason to doubt it—then it seems pretty clear that each of the branchial clefts has a lining of ectoderm.

The pharyngeal rudiment behind the fourth branchial pouch tapers off to a solid cord of cells, the diameter of which gradually diminishes. Since the pericardial rudiment only extends to the commencement of the œsophagus, the alimentary canal beyond this point, while having no organic continuity with, yet lies directly over, the great yolk-mass. The differentiating stomach, presumably from lack of space in a ventral direction, expands laterally to the left. The dorsal mesenteries are for the same reason not vertical and median, but more or less horizontal. The liver appears at this stage as a solid lateral (to the left) outgrowth of the alimentary canal. On the right, dorso-lateral in position, is the dorsal pancreatic rudiment, solid, surrounded by and passing without any sharp boundary into the surrounding mesenchyme, which forms the spleen rudiment. This latter arises in connection with venous blood, which is part of the liver and yolk-sac circulation. It will be seen in the section on the vascular system that the subintestinal vein breaks up into capillaries on the yolk-sac, and that as regards circulation the liver forms part of the yolk. The spleen, then, has the usual teleostean connection with the blood of the subintestinal vein.

In Stage 25 the operculum—a typical teleostean one—has grown backwards to the level of the second gill-arch, and the gill-cavity begins to differentiate. Chinks appear in the solid cleft rudiments separating apart the flat epithelium lining of the two sides of each slit. The arches are not at this stage the long curved structures which they eventually become; almost the whole of one arch is visible in one transverse section. The branchial rudiment no longer projects to the exterior as it did in earlier stages. A study of Pl. 3, fig. 20, a horizontal section through an embryo of Stage 25, will show that the part of the branchial rudiment not yet covered by the operculum is overlaid by the tissue masses of the body wall, what is equivalent to the posterior wall of the last cleft extending forwards so as to overlap the cleft rudiments and being continuous with the backwardly growing operculum. No space exists between the surrounding tissues and the three hinder arches, but between the backwardly growing operculum and the dorsal part of the first gill-arch a space is being hollowed out and lined by epithelium. The chinks in the solid cleft rudiments already described and this small space represent the opercular cavity at this stage. There is no communication between it and the exterior.

The liver shows signs of histological differentiation, its cells no longer resembling those of the alimentary canal, but being distinguishable from the latter by their paler staining properties. Blood-corpuscles are plentiful in the developing liver. The spleen is bulky.

Posteriorly the alimentary canal passes into a dilatation which receives the kidney ducts. This is the cloaca.

In Stage 26 hyomandibular and hyoid arches are more clearly visible in sagittal sections. The gill-arches have increased in length, and are, therefore, more curved; the operculum has grown further back. There are now two small openings behind its posterior edge, which is at about the level of the second gill-arch, dorso-lateral in position, leading into the small differentiating opercular cavity. They each represent a small dorsal portion of the lateral opercular opening such as is found in many teleosteans. Ventrally the cavity is not yet hollowed out. A transversely directed slit-like lumen commencing behind the level of the hyoid separates roof from floor in the developing pharynx. The œsophagus is solid, but the rest of the canal is gradually acquiring a lumen.

The dorsal pancreas more or less surrounds the alimentary canal and one lobe has grown tailwards for a distance of 180 μ (Pl. 3, fig. 21). The spleen has now attained to a diameter greater that that of the stomach. The bile-duct and gall-bladder rudiments differentiate at this stage out of the solid stalk connecting liver and alimentary canal. The lumen of the bile-duct develops from the alimentary canal outwards.

In the next stage (27) the formation of the tongue has begun. Its adult relations are illustrated by Pl. 3, fig. 22, there being nothing of special interest in its development. The gill-cavity is still small though the branchial apparatus has elongated considerably. The thymus rudiment first occurs here. It develops on the outer dorsal side of the four branchial clefts, 2, 3, 4, and 5.

The ventral pancreatic rudiments are solid outgrowths from the bile-duct. They meet the dorsal pancreatic rudiment so that at its hinder end the stomach is almost surrounded by pancreas. The adult pancreas would seem to be mainly the derivative of these ventral rudiments. The gall-bladder, originally solid, is being hollowed out. The spleen is very conspicuous. The temporary cloaca has become quite flattened out.

Stage 29.—A median sagittal section at this stage shows, in the constriction marking the origin of the tongue, the rudiment of the thyroid which is a solid derivative of the floor of the pharynx. (In Pl. 3, fig. 22 this gland is seen at Stage 34.) The mouth is not yet open, but the rest of the canal has a lumen. The bulbus lies directly under the second gill-arch. In Stage 29 there is the first trace of respiratory tissue. The two pore-like openings into the gill-chamber are shown in Pl. 4, fig. 23—a transverse section through this stage. The pancreas is very much invaded by the mesenchymatous vascularised masses which constitute the spleen rudiment. The posterior part of the liver widens out so as to cover in the dorsal part of the yolk, both right and left.

The alimentary canal has almost reached its adult structure in Stage 30. The larva is now capable of feeding and makes frequent swallowing movements, the respiratory lamellæ on the gill-arches being well developed. The heart lies posterior to the gill-chamber now (compare Pl. 4, fig. 23), and an opening from the latter on the ventral and ventro-lateral surface of the creature has been burrowed out. This is continuous into the dorso-lateral pore-like openings of the preceding stages, and is the functioning "exit" from the gill-cavity already described in Part I (compare Pl. 3, fig. 19). Later on the lateral portions become obliterated and the single median ventral opening is the result.

The alimentary canal of Symbranchus is straight, running from mouth to anus without bend or curve, showing little change in diameter, and very little to distinguish externally the different parts, at any rate behind the pharynx. But on dissecting open the canal of the fully adult Symbranchus, the surface appearance of the interior shows quite clearly œsophageal, stomach and intestinal areas. The sections at Stage 30 and onwards show that the stomach is of slightly wider calibre than the œsophagus, and that the characteristic macroscopic appearance is due to the presence of glands. Externally and microscopically it is possible to distinguish cardiac from pyloric part of the stomach, the latter having stout muscular walls. A pyloric valve, which, however, only begins to differentiate in Stage 32, connects the pylorus with the duodenum. The inner lining of the œsophagus and stomach is thrown into longitudinal folds, in sharp contrast to the intestine, which is honeycombed.

The thymus at this stage is very conspicuous in both sagittal and transverse sections. The separate rudiments have all joined to form one large gland.

In a median sagittal section the thyroid is distinguishable as a rectangular band of cells, stretching backwards from the root of the tongue to the first gill-arch. Transverse sections show that anteriorly the thyroid bifurcates. The structure at this stage is not glandular. The liver is like that of the adult, and the gall-bladder is walled in by two layers of flat epithelium.

At Stage 32 the pyloric valve is differentiated. The pyloric part of the stomach projects back like a spout into the duodenum. Finger-like protuberances of the latter are packed all round the anterior part of this spout, thus hiding the constriction that would otherwise exist between the pylorus and duodenum. The blind, pocket-like outpushings, mostly on the ventral side in the beginning, can easily be seen at this stage, growing forward more or less parallel with the pyloric part of the stomach, in the mesodermal covering of the canal. (In Pl. 4, fig. 24 a transverse section through the valve at Stage 35 is given.)

The special character of the pyloric valve just described and the study of its development suggests one or other of the following hypotheses :

(1) That these finger-like outpushings of the duodenum represent degenerating pyloric cæca, or—

(2) That pyloric cæca have been evolved by an elaboration of these cæcal outgrowths consequent upon their becoming free from the muscular coat of the alimentary canal.

An examination into the condition of pyloric cæca in Teleosteans seems to furnish evidence in favour of either interpretation. Many Teleosteans are without pyloric cæca, and the number of these cæca when present and their arrangement is extraordinarily varied.

Among Ganoids, Acipenser, the most primitive, is possessed of pyloric cæca. It is interesting to note that the early appearance of the cæcal rudiments, as described by A. Nicholas (7), recalls the adult condition of the pylorus arrangements in Symbranchus, only, that in the case of Acipenser, the outpushings of the duodenum, while directed towards the stomach, do not fuse with the walls of the latter —the muscular coat of the alimentary canal is involved in the outpushing.

In Amia the pyloric cæca are absent, but the pyloric valve, as described by Piper (8), resembles quite closely that of Symbranchus.

Among Teleosteans the pyloric valve of the eel, where pyloric cæca are absent, is of the Symbranchus type.

Pyloric cæca are absent in Cyprinoids, Labridæ, Gobiiæ, Blennidæ, Syngnathus and Cobitis—forms in which there is no obvious stomach. In Blennius pholis and Carassius auratus there is no specialisation of the pyloric valve, there being just a simple ring-shaped opening, but there is a long, very much coiled intestine. If there is a similar absence of a specialised valve combined with the presence of a very long intestine in the other members of the above-mentioned orders, then it would seem as though the absence of pyloric cæca and any specialisation of the pyloric valve in these Teleosteans were compensated for by the presence of a very long intestine.

A comparison of such forms as Cottidæ with one of the Catosteomi (Stickleback) is instructive as illustrating how the degeneration of pyloric cæca (according to Goodrich (**3**) pyloric cæca are lost, as a rule, in this suborder) may possibly bring about an appearance such as that described in Symbranchus. In Cottus the short finger-like processes lying round the front part of the duodenum, with their blind ends lying on the stomach, form a circle of pyloric cæca. A dissection of this part of the alimentary canal reveals a rosette-like valve (the protrusion of the stomach into the duodenum) surrounded by the individual openings of the blind sacs.

At first sight there is no constriction visible between the stomach and intestine in the stickleback (Gasterosteus aculeatus), but the constriction is really present though hidden by two broad-mouthed blunt bulgings of the duodenum in the direction of the stomach. One of these pockets is bigger than the other as a rule (in one specimen examined the smaller of the two was very small). Both are well bound down to the alimentary canal by connective tissue—in fact, they seem to show a tendency to fuse with it. The valve is of the same type as that of Symbranchus, so that, should the pocket-like outpushings become fused with the alimentary canal, then the likeness between the pyloric arrangements of the stickleback and those of Symbranchus would be perfect.

In this case it would seem that, if the pyloric cæca are really disappearing in the Catosteomi, then the Symbranchus pyloric valve is the result of degeneration of pyloric cæca—an indication of specialisation in Symbranchus.

Against such a theory, however, it must be remembered that this form of valve occurs in Lung-fishes as well as in Amia, and that it is more primitive in nature than cæca, a quite similar valve developing in other cases (cf. various insects).

Stage 35, etc.—Little more remains to be said. The pyloric valve is fully developed in Stage 34. In Stage 36 the mouth is provided with unicellular glands and numerous papillæ. A rectum can also be distinguished, being of slightly wider diameter than the rest of the intestine and its commencement being marked by a sphincter muscle.

The yolk-mass gradually diminishes, being pushed into a dorso-lateral (right) position by the encroachment of the developing viscera, the subintestinal vein being, however, always in close connection with it. To the end of its existence it is always surrounded by a yolk-syncytium.

Thymus and thyroid increase in size. The former is a dense mass of deeply staining units interspersed with blood-corpuscles and is a conspicuous object both in transverse and sagittal sections. At the hind end of the thyroid a central lumen filled with a structureless colloidal substance is surrounded by cells. In the adult condition the liver is elongated and unilobed. Its anterior end stretches almost to the pericardium, and is on the left side of the alimentary canal, but together with the great length there is a slight twisting of the long lobe, so that for part of its course this lies ventral to the alimentary canal, while its hind part (which reaches almost to the anus) is on the right of the intestine.

The gall-bladder is large and conspicuous. It lies in the liver, its ventral surface being just flush with that of the liver.

The adult pancreas is a comparatively compact and very much elongated gland. It reaches anteriorly to the gall-bladder, circumscribes the alimentary canal at the pylorus, in which place it attains its greatest bulk, gradually dying out in the hinder intestinal regions. The anterior part is dorso-lateral, the posterior part beyond the pyloric valve ventro-lateral, both anterior and posterior parts lie between the alimentary canal and the liver (Pl. 4, fig. 24).

The spleen is a compact, lenticular-shaped structure more posterior than the gall-bladder, lying near the liver to the right of the intestine.

Yolk and Alimentary Canal.

In Symbranchus, as elsewhere, in the early stages, centres of growth are characterised by the presence of yolk, which is being reduced to fine dust-like particles by the activity of the intermingled protoplasm. In Stages 18, 21 and 22 large quantities of such yolk in process of assimilation with large, deeply staining yolk-nuclei are to be found under the hind end of the embryo. As the embryo increases in size a strand of this yolk-syncytium, with its characteristic nuclei, extends into the free tail part of the embryo immediately over the differentiating subintestinal vein. Pl. 4, fig. 25 is a sagittal section through an embryo of Stage 24. A somewhat similar condition of things is described by Assheton (2) for Gymnarchus niloticus. Similarly in later stages an anterior plug is visible (Stages 29, 30 and 31) behind the pericardium. This plug (Pl. 4, fig. 26) in Stage 31 becomes more or less separated from the rest of the yolk, and has much the appearance of a group of round and highly reticulate large nucleated egg-cells. These cells lie in the mesenchyme immediately posterior to the pericardium and ventral to the hinder part of the œsophagus.

Pharyngeal Pouch.

The whole of the lining of the gill-chamber of Symbranchus as well as the roof and floor of the mouth is provided with a rich network of blood-vessels forming a respiratory tissue. Anterior to the first efferent branchial the aortic roots of each side give off a large artery which breaks up into innumerable capillaries in the roof of the mouth. This blood is drained back into the anterior cardinals.

Reference was made in the introduction to a "pouch" or "pocket" given off from the roof of the mouth. Traces only of this "pouch" can be found in the young creature, but sections through the adult show that it is an invagination of the lining of the gill-cavity, probably a device for increasing the area of the respiratory surface. This invagination begins immediately behind the attachment of the first branchial arch to the pharynx, and the pocket so formed extends backwards with a gradually decreasing diameter to the attachment of the fourth arch. The opening into the pocket is large, longer than broad.

(B) The Excretory System.

Solid pronephric nephrotomes are discernible about Stage 18.

There is little to distinguish the rudiment of the pronephros from that of its duct. The nephrotome of the third trunk myotome appears to give rise to the functional pronephros, those of succeeding segments forming the archinephric duct. It is not possible to say whether other segments besides the third have any share in the formation of the pronephros, but in the light of subsequent events it does not seem likely.

The separation, which begins at Stage 20, of the various nephrotomes from their respective segments to become converted by a process of fusion into an archinephric duct seems to take place practically simultaneously—the hinder parts of the duct being formed as soon as the anterior portions.

Stage 21.—The third nephrotome has become more deep-seated. Although still solid it has increased in size because of the development of lacunæ, these lacunæ being the first indication of the pronephric chamber. The archinephric duct rudiment is still solid, round and cord-like, lying approximately on a level with the dorsal aorta.

Stage 24.—The rounded pronephric chamber rudiment is now being converted into a typical Malpighian capsule, and the formation of a glomerulus from the dorsal aorta has begun. In its development and in its fully formed condition this pronephric chamber exactly resembles the Malpighian capsules of the mesonephros, though the former is larger than the latter. The glomerular rudiment is formed of deep-staining cells interspersed with blood-corpuscles, the whole forming a little clump lying in the cavity of the pronephric chamber, the lining of which is exceedingly thin. This clump lies on that side of the chamber which is nearest to the dorsal aorta rudiment.

The cells of the archinephric duct rudiment are assuming an epithelial character, and in some places there is a lumen.

Both ducts have become more deep-seated and now lie ventral to the dorsal aorta. The inter-renal vein appears at this stage.

Stage 25.—The pronephros has increased greatly in size, and the two pronephric chambers with their respective glomeruli are very conspicuous structures in horizontal sections. They lie in contact with the paired aortic roots just as these are joining up to form the dorsal aorta. An opening from the short pronephric tubule into the archinephric duct is established, the duct being now perforate throughout its length.

Stage 26.—The pronephros has reached its maximum size. The glomerulus of each chamber is conspicuous, and the opening into the tubule is funnel-shaped. There is no obvious boundary between tubule and duct. Anteriorly the latter is becoming coiled, posteriorly, it is quite straight. Its opening into the temporary cloaca has already been mentioned in Part I.

In Stage 26 the pronephros has become more deep-seated, lying immediately ventral to the dorsal aorta, which at this point gives off a cœliaco-mesenteric artery behind the aortic roots. The archinephric duct is surrounded by blood, the posterior cardinals anteriorly, and the inter-renal vein posteriorly, bathing the ducts on their inner side, while on the outer side is blood which communicates with the segmented somatic veins. There is no sign of the "pseudo-lymphatic" tissue except at the extreme anterior end of the archinephric duct, where a patch of deeply staining mesoderm may possibly represent this tissue.

Stage 27.—At this stage the glomeruli are supplied from the cœliaco-mesenteric artery, the pronephros being quite ventral to, and much deeper than, the dorsal aorta. The "pseudo-lymphatic" tissue has increased, and is no longer confined to the anterior end of the kidney rudiment. The tissue at this stage consists of scattered masses, deeply stained, lying in the neighbourhood of the ducts and blood-vessels.

Stage 28.—The archinephric duct is more coiled and the pronephros has become more posterior relative to the pectoral fins. The urinary bladder has increased in length.

Stage 30.—At this stage the larva is capable of feeding and is breathing by means of its gills. Since the first rudiments of the mesonephros are barely distinguishable in Stage 29, it seems probable that the pronephros is the functional kidney at this time. The "pseudo-lymphatic" tissue has increased in bulk, and the rudiments of the mesonephros are larger than in the preceding stage. They are segmental structures beginning in the twenty-fifth segment, i. e., in the region of the posterior half of the archinephric duct, the anterior half being quite free from mesonephric tubule rudiments. They consist of small, solid knob-shaped masses of deeply staining cells quite readily distinguishable from the surrounding "pseudo-lymphatic" tissue lying mostly dorsal to the archinephric duct. Although they seem to develop outwards from the immediate neighbourhood of the duct, indenting its otherwise straight course, yet they are not formed from the cells of the duct, but appear to be condensations of mesenchyme. They rapidly increase in size, become moulded into solid twisted structures of the nature of solid cords, and eventually acquire a lumen secondarily. They extend backwards to the commencement of the urinary bladder, through eighteen segments, i.e., to the forty-third post-occipital myotome.

Stage 32.—The mesonephric tubules have become hollow in most cases, and the cells surrounding the lumen have a more epithelial character. The slightly club-shaped end of each tubule is hollowed out into a rounded cavity very like that seen in the development of the pronephros, and a mass of darkly stained mesenchyme in the neighbourhood of each of these Malpighian capsule rudiments, probably represents the rudiment of the glomerulus.

Stage 33.—Many mesonephric tubules have acquired an opening into the archinephric duct which is distinguishable from the former by its paler staining powers. The Malpighian capsules in many of the segments are fully formed—the glomerular artery arising from the dorsal aorta by a common root with an intersegmental artery (Pl. 4, fig. 27).

Stage 34.—Horizontal sections at this stage show the segmental character of the mesonephros at its best. By this time all the mesonephric rudiments have fully developed and Malpighian capsules with their glomeruli and tubules occur, one in each segment. The urinary bladder has lengthened out and the pseudo-lymphatic tissue has increased.

Stage 35.—Although externally the creature is quite adult in appearance the mesonephric tubules have not yet attained their complete development. They are still readily distinguishable from the duct by their staining reaction, the cells of the duct being much redder in preparations stained with eosin and hæmotoxylin than the purple ones of the tubules.

The very much coiled anterior end of the duct is embedded in a capsular structure formed of "pseudo-lymphatic" tissue, the elements of which are smaller and more densely packed than in the earlier stages. Posteriorly the tissue has to make way for the tubules, and is not so abundant.

Stage 36.—The epithelial lining of the tubules has now quite an adult appearance. New secondary tubules are beginning to form between the earlier tubules. These new rudiments repeat the developmental history of the first formed units, being closely packed, densely stained tissue masses in the immediate neighbourhood of the duct when they first appear. Eventually they become coiled tubules. However, it is very difficult to follow their history completely. These secondary tubules are not numerous in Symbranchus.

Stage 43.—The pronephric chambers with their glomeruli are still present, though insignificant structures, very much crushed out by the surrounding tissues. Their position is more posterior relative to the shoulder girdle. New mesonephric tubule rudiments are still being formed.

The urinary bladder reaches anteriorly almost to the commencement of the rectum, and posteriorly a blind sac-like dilatation protrudes into the tail.

The Adult.—A dissection of the excretory system of an adult of twelve inches shows an elongated paired kidney lying on either side, and in close contact with the inter-renal vein. It extends from a point lying dorsal to the middle of the pericardium—to the rectum—and is flattened dorso-ventrally. At the beginning of the rectum (thirty-eighth trunk-segment) it opens into the urinary bladder, which has the same diameter as the rest of the excretory organ. A small diverticulum of the bladder passes into the tail. The urinary opening is distinct from, but immediately posterior to, the anus.

(c) Vascular System.

Rudiments of the dorsal aorta appear in Stage 19 as clear spaces lying between the nephrotomes and notochord. In Stage 21 the dorsal aorta, in which blood-corpuscles are beginning to appear, is still paired throughout its length.

A sagittal section through Stage 22 shows that the blood from the yolk collects into a widened receptacle and then passes into a single and almost straight elongated vessel which expands somewhat just where it comes into connection with the first two visceral arches. The widened receptacle, the rudiment of the atrium, is quite anterior to the embryo. The rudiment of the ventral aorta communicates with that of the dorsal aorta by vessels which run up the mandibular and hyoid arches respectively. The blood reaches the yolk-sac by the subintestinal vein, a large vein which is situated in the ventral part of the mesenchymatous tissue underlying the alimentary canals (*s. i. v.*, Pl. 4, fig. 25). This vein first appears in that part of the "tail" which is nearest the yolk-sac, and gradually extends backwards as the "tail" elongates.

Stage 24.—The straight tubular heart is twisting and the rudiment of the bulbus first appears. The ventral aorta is exceedingly short and topographically posterior to the ventricle, on account of the position of the pericardial rudiment. (Pl. 1, fig. 11, shows the ventricle.) The vessels in the branchial arches are making their appearance, though not yet connected up with the ventral aorta.

The anterior cardinals, ducts of Cuvier, and veins of the fin are appearing, in the form of clear spaces in the mesenchyme provided in some cases with blood-corpuscles, while in the tail a caudal vein is visible. This caudal vein some little distance anterior to the anus divides up into two branches, one of which forms the inter-renal vein, and dies out anteriorly at this stage; the other, descending to the right of the alimentary canal, forms the subintestinal vein already mentioned (*s. i. v.* Pl. 1, figs. 9, 10, and Pl. 2, fig. 13). This subintestinal vein breaks up in the yolk into a number of capillaries which drain into two large vitelline veins, which in turn form by their union the sinus venosus.

Two large vessels are given off to the pectoral fins from the dorsal aorta in the neighbourhood of the pronephros. Posterior to the pronephros, the dorsal aortæ, until now distinct, are fusing up to form a single vessel.

Stages 25 and 26 (Text-fig. 2).—The bulbus lies immediately ventral to the first branchial arch, so that the first branchial artery has a vertical direction, and the ventral aorta must take a posterior direction in order to supply the three other branchial arches. This backwardly directed part of the ventral aorta is paired. A smaller branch from the bulbus, the remnant of the disappearing hyoid aortic arch, runs forward to the hyoid arch, but it is quite insignificant compared with the four aortic arches, which practically convey all the blood from the heart to the dorsal aorta. The mandibular aortic arch has completely disappeared. The anterior ends of the aortic roots are prolonged forward to the head as dorsal carotids, and each of these is joined by a vessel from the hyoid arch. A cœliaco-mesenteric artery is present, as has already been explained in connection with the excretory system.

Text-fig. 2.

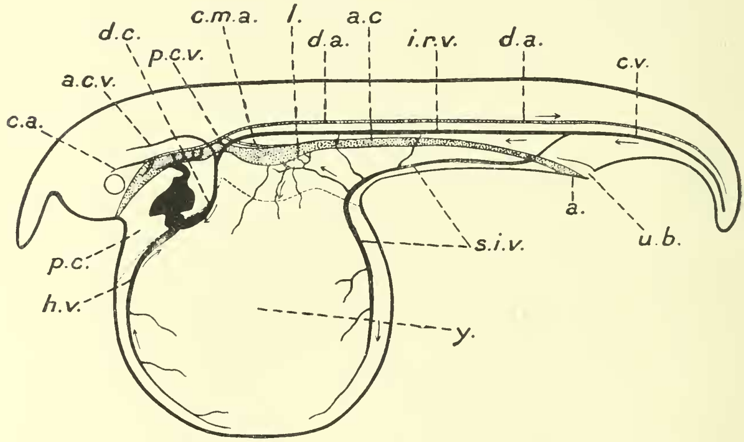


Diagram of the vascular system of a larva of Stage 26. *a.* Anus. *a. c.* Alimentary canal. *a. c. v.* Anterior cardinal vein. *c. a.* Carotid artery. *c. m. a.* Cœliaco-mesenteric artery. *c. v.* Caudal vein. *d. a.* Dorsal aorta. *d. c.* Ductus Cuvieri. *h. v.* Vitelline vein. *i. r. v.* Inter-renal vein. *l.* Liver. *p. c.* Pericardium. *p. c. v.* Posterior cardinal vein. *s. i. v.* Subintestinal vein. *u. b.* Urinary bladder. *y.* Yolk.

At this stage the anterior cardinals are fully developed. The ductus Cuvieri are long, since the sinus venosus is still topographically the most anterior part of the heart. The vitelline vein enters the sinus anterior to the ductus Cuvieri. The paired portions of the posterior cardinals, now fully formed, are very short, the inter-renal vein commencing in the seventh segment. Posteriorly, especially in the neighbourhood of the developing urinary bladder, however, the inter-renal vein shows its double nature. Blood from the yolk-mass bathes the tissue of the liver, which, as regards its circulation, may be considered as part of the yolk. The large veins from the fin become connected up with the ductus Cuvieri.

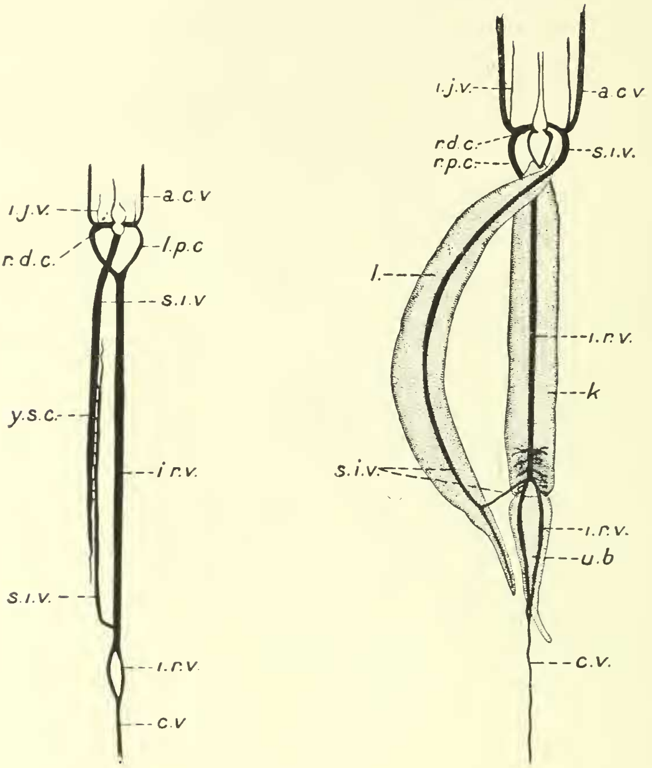
Stage 26.—The hyoid aortic arch has almost disappeared. The four branchial aortic arches are large. The heart is becoming relatively more posterior and more twisted. There are many connections between the inter-renal vein and the sub-intestinal vein, which do not show any segmental arrangement. Inferior jugulars now appear, and the right posterior cardinal is becoming larger than the left one.

Stages 27 and 28.—The heart is now in such a position that the bulbus lies under the third gill-cleft, so that the ventral aorta has to run forwards to the first and second afferent branchial arteries and backwards to the third and fourth. A small branch of the ventral aorta to the hyoid arch still persists. In Stage 30 this has disappeared. The aortic roots join up to form the dorsal aorta posterior to the fourth branchial arch, the fourth branchial efferents being almost at right angles to the dorsal aorta. Anteriorly the aortic roots are continued forwards as the dorsal carotids, which receive the efferent end of the hyoid aortic arches. Near the infundibulum the carotids divide up into internal and external branches, which are distributed, the former to the brain, the latter mainly to the eyes and nose. The inferior jugulars which collect the blood from the lower jaw and the parts below the ventral aorta, and which are asymmetrical, the right being smaller than the left, pass between the bifurcating anterior ends of the thyroid. The ventricle is now topographically the anterior part of the heart.

Stage 29.—Text-fig. 3A shows the principal veins at this stage and needs no description.

Stage 30.—The vascular system is practically in its adult condition at this stage. Sinus venosus, atrium, ventricle and bulbus have assumed their adult topographical relations, though all these structures are not relatively so elongated as in the adult Symbranchus, nor does the heart occupy such a posterior position. The ventral aorta, moreover, is not the long structure it is in the adult. The heart shows the ordinary Teleostean features (compare Pl. 4, fig. 26). An

Text-fig. 3.



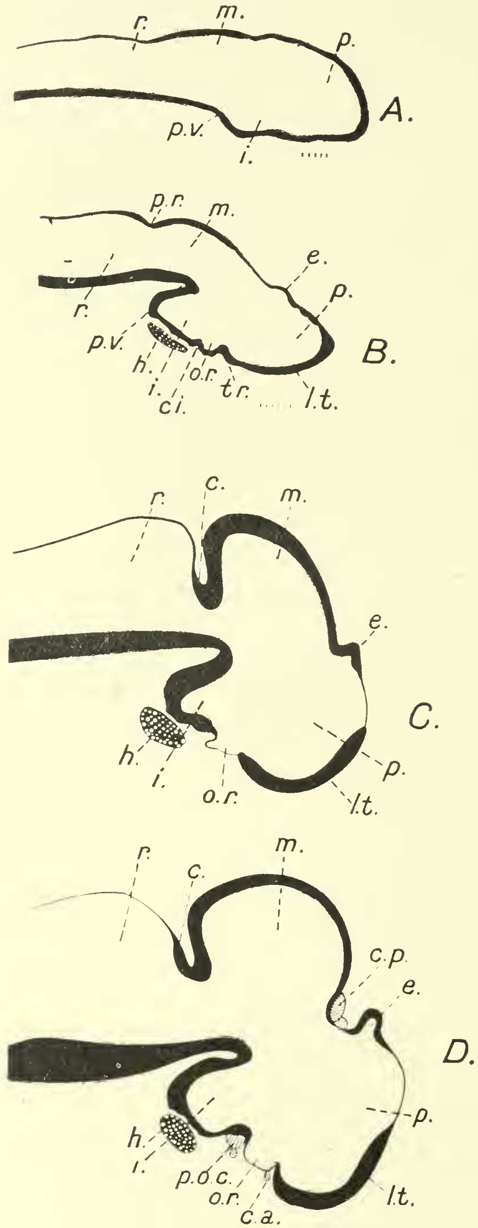
A. B.

A. Diagram of the principal veins of a larva of Stage 29 (ventral view). B. Diagram of the venous system of adult Symbranchus (ventral view). The liver has been pinned out to the right. *a. c. v.* Anterior cardinal vein. *c. v.* Caudal vein. *i. j. v.* Inferior jugular vein. *i. r. v.* Interrenal vein. *k.* Kidney. *l.* Liver. *l. p. c.* Left posterior cardinal. *r. d. c.* Right ductus Cuvieri. *r. p. c.* Right posterior cardinal. *s. i. v.* Subintestinal vein—vitelline vein—hepatic vein. *u. b.* Urinary bladder. *y. s. c.* Yolk-sac circulation.

artery is given off from the dorsal aorta to supply the urinary bladder. Intersegmental arteries are also given off to the body-segments from the dorsal aorta (Pl. 4, fig. 27), while segmental veins convey venous blood from the muscles into the blood sinus which bathes the excretory organs. The inter-renal vein is double in the neighbourhood of the urinary bladder, this structure lying between the two branches; the right blood-vessel is always the larger.

The free portion of the left posterior cardinal (compare Text-fig. 3A) is reduced in size at this stage; eventually it atrophies completely, the blood of the inter-renal vein being conveyed to the heart by the right posterior cardinal. The vitelline vein, instead of opening ventrally into the sinus venosus, has its opening shifted well to the left so that it opens into the ductus Cuvieri symmetrically with the large right posterior cardinal (Text-fig. 3B). It has been shown in the earlier stages that the liver is practically a part of the yolk as regards its circulation. It has also been shown that the caudal vein is continued forwards as an inter-renal (which, however, is a paired vessel in the neighbourhood of the urinary bladder) and a subintestinal vein. This division of the caudal vein takes place at the anterior end of the urinary bladder. In the earlier stages the subintestinal vein breaks up in the yolk into a number of capillaries which unite to form a large vitelline vein. However, as the creature grows, and as more and more of the yolk-mass becomes included in the normal contour of the animal, the subintestinal vein never loses its individuality, so to speak, but can be traced forwards through the liver, becoming the hepatic vein. This vein gradually increases in diameter as the pericardium is reached, because it receives blood from the yolk and liver. As the creature grows and as the liver elongates blood communications between kidney and liver are also set up. This is very striking in Stage 30, when the subintestinal vein gives off a large branch, which, running ventral to the alimentary canal, enters the posterior end of the liver and there breaks up into capillaries. Later on more blood passes into the liver from the hinder parts of the creature, this blood eventually finding its way into the anterior parts of the sub-

Text-fig. 4.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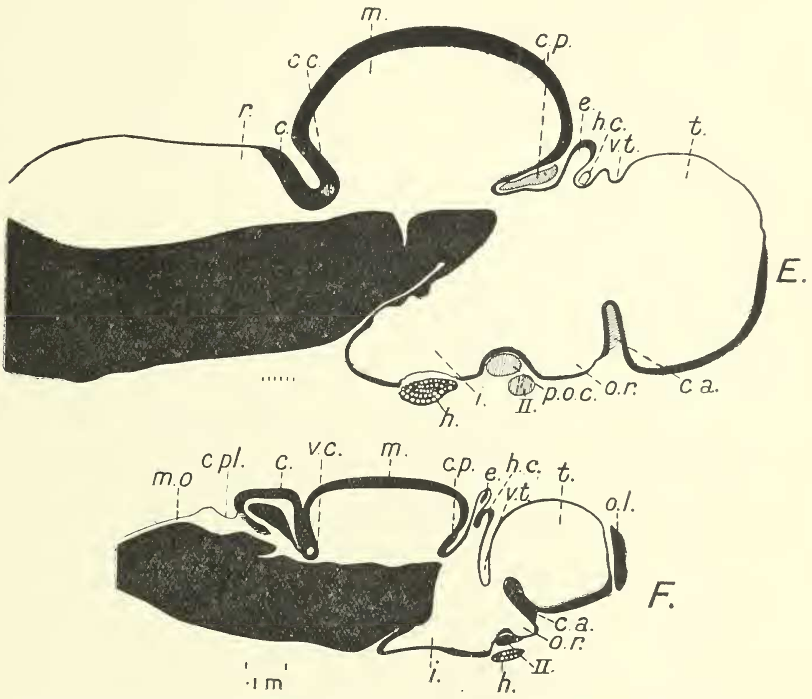
intestinal vein, the front end of which is the original vitelline vein. Even when the yolk has quite disappeared this large subintestinal vein, can be traced right up to the liver. The

TEXT-FIG. 4—continued.



Sagittal sections through brain of Symbranchus. A. Stage 22. B. Stage 24. C. Stage 26. D. Stage 28. E. Stage 30. F. Stage 34. *c.* Cerebellum. *c. a.* Commissura anterior. *c. c.* Cerebellar commissure. *ci.* Optic chiasma. *c. p.* Commissura posterior. *c. pl.* Choroid plexus. *e.* Epiphysis. *h.* Hypophysis. *h. c.* Habenular commissure. *i.* Infundibulum. *l. t.* Lamina terminalis. *m.* Mesencephalon. *m. o.* Medulla oblongata. *o. l.* Olfactory lobes. *o. r.* Optic recess. *p.* Prosencephalon. *p. o. c.* Post-optic commissure. *p. r.* Rhombo-mesencephalic fissure. *p. v.* Primitive brain fold. *r.* Rhombencephalon. *t.* Telencephalon. *tr.* Torus transversus. *v. c.* Valvula cerebelli. *v. t.* Velum transversum. II. Optic nerve.

subintestinal vein is thus seen to pass directly into the hepatic vein of the adult, and its junction with the kidney vessels posteriorly recalls the posterior vena cava in Polypterus. (**6**).

(D) Nervous System.

The Brain.

The course of events in the conversion of the flat, wide-spreading medullary plate into the solid keel-like neural rudiment, and the formation, out of the anterior part of this plate, of a brain-rudiment with ventricles which develop secondarily, is quite Teleostean in character. Except for a slighter wider diameter and the presence of optic vesicles there is nothing to distinguish the brain from the spinal cord in the stages up to 20. In a dorsal view of Stage 22, however, the three ventricles are discernible, while sagittal sections of about this age (Text-fig. 4 A) show that the floor of the archencephalon is becoming delimited posteriorly by an uprising of the brain floor.

Cranial flexure has increased considerably by Stage 24 (Text-fig. 4 B), when the ventricles are very well defined. The mesencephalon and rhombencephalon are conspicuous objects in a dorsal view of the whole embryo (Pl. 1, fig. 7), a somewhat narrow portion connecting the latter with the former. Indications of a cerebellum rudiment are discernible, as two thickenings on the anterior wall of the rhombencephalon cavity. In the prosencephalon a pineal rudiment is distinguishable. The optic recess is somewhat narrow.

Cranial flexure continues to increase (Text-fig. 4 C), reaching a maximum in Stage 28 (Text-fig. 4 D). By this time (Stage 28) nerve-fibres have appeared. The posterior commissure and habenular ganglia as well as anterior and post-optical commissures are formed. The paired thickenings alluded to above have grown backwards and inwards to form a rudimentary cerebellum, while on either side of the lamina terminalis the basal thickenings are converting the prosencephalon into a solid structure with a thin non-nervous roof.

By Stage 30 (Text-fig. 4 E) the brain has begun to straighten out and to assume much of its adult appearance in transverse sections. The rudiment of the velum transversum is present and olfactory lobes are developing. The infundibulum is closely apposed to the floor of the hind brain, which latter is becoming very solid. In addition to the commissures already present the cerebellar commissure has now appeared, a torus longitudinalis is distinguishable, and the fibres of the optic nerve are connected up with the roof of the mid-brain, as is usual.

There is no fundamental difference between the brain of Stage 34 (Text-fig. 4 F) and that of the adult. The brain is by now (Pl. 4, fig. 28) very solid, the once large ventricles being almost obliterated. The paired olfactory lobes are conspicuous at the anterior end of the telencephalon, while ventrally the lateral lobes of the infundibulum have greatly increased in size. The cerebellum and valvula cerebelli are also much larger, while the tuberculum acusticum and the vagus lobes have almost obliterated the once large fourth ventricle.

The brain dissected from an adult Symbranchus is characterised by a more perfect separation of the chief brain parts one from the other. These are very close together in Stage 34. Another difference is that the optic lobes are not relatively so large as they were. The telencephalon, on the contrary, is larger, while the cerebellum and valvula cerebelli are much more important than they were at Stage 34. The sacculus also develops late.

Cranial Nerves.

The cranial nerves of the adult Symbranchus as well as their respective branches are quite typical.

The eye-muscle nerves are small, like the eye itself, and the olfactory and optic nerves are long because of the general elongation of the head.

The developmental history of the cranial nerves is, briefly, as follow:

Stage 21.—The rudiments of the ganglia of the fifth, seventh and tenth nerves are visible.

Stage 24.—Nerve-fibres have appeared in the peripheral parts of the brain and the spinal cord, and the roots of the fifth, seventh, eighth, ninth and tenth can be distinguished. These roots are quite distinct one from the other.

Stage 25.—The large roots of the fifth, seventh, eighth, ninth and tenth are conspicuous objects in sections, the fifth and seventh, in contra-distinction to their adult condition, being separated by a considerable interval. The optic and olfactory nerves are possessed of fibres.

Stage 26.—The third nerve is developing. On account of the cranial flexure this nerve appears before the optic nerve in transverse sections.

Stage 28.—The fourth nerve can be traced from the junction of the optic lobes with the rhombencephalon. The acustico-lateral ganglionic elements of the seventh nerve can be easily distinguished from the Gasserian ganglion in these early stages and the lateral line root is widely separated from the vagus root. Even in the adult these two roots are separate, the lateral line branch transversing part of the floor of the auditory capsule in order to reach the vagus ganglion.

The three main branches of the fifth can be traced to their respective destinations in Stage 28 and the hyomandibular branch of the seventh is conspicuous. The rudiments of the pre- and post-trematic branches of the ninth and tenth nerves are distinguishable in sagittal sections, as well as the palatine branch of the ninth.

Stage 30.—Gasserian and geniculate ganglia are becoming massed into one large ganglionic centre which lies outside the brain-capsule—the condition which obtains in the adult. The pre- and post-trematic branches have fibres. By Stage 32 buccal and palatine branches of the seventh as well as ophthalmic can be traced.

Spinal Nerves.

Fibres can be detected in the spinal nerve-rudiments in Stage 24, although the nerve is mostly protoplasmic at this stage. The nuclei, which later on will form the nerve-sheath, lie more or less scattered round this protoplasmic-like developing nerve.

In Stage 26 these nerve-sheath nuclei, which have been derived from the mesenchyme separating spinal cord from myotome, are more closely apposed to the nerve-rudiment, which is now much more strongly fibrous and has almost entirely lost its protoplasmic character. In stages beyond 26 the nerves have the usual adult structure.

IV. Summary.

(a) General Features of Development.

(1) The egg of Symbranchus is small, its development typically Teleostean and rapid, the larva hatching out in about seven days at a tropical temperature.

(2) A rostrum appears just before the larva hatches, increases in size, attains a maximum length of about 1 mm. when the creature is 7 mm. long, decreases in size, gradually dying down to a rounded pad, and eventually disappears just before the adult stage is reached.

(3) The larva possesses pectoral fins and the shoulder girdle persists in the adult. These fins appear early, are muscularised by the first three trunk myotomes and innervated by the first three spinal nerves. They develop rapidly, reach their maximum size seven or eight days after hatching, shrivel somewhat, and then drop off bodily at Stage 34. The pectoral fins are mainly respiratory organs and possess a rich network of capillaries. There are three principal blood-streams in the fins—one central, afferent, two marginal, efferent.

The establishment of perfect branchial respiration is coincident with the falling off of the fins, i. e. when the creature is ten days old.

(4) No trace of pelvic fins has been found.

(5) Perforated gill-slits of the Elasmobranch type do not occur in early stages, the clefts only becoming perforate after

they are covered by the operculum. When branchial respiration is just beginning the gill-chamber opening is a single crescent-shaped one; as development proceeds the arms of the crescent are gradually obliterated, owing to the fusion of the backwardly growing operculum with the body-wall, and a single median ventral opening is the result.

(6) There is a blind diverticulum in the dorsal roof of the mouth behind the hyoid.

(B) Alimentary Canal.

(1) The alimentary canal has a typical Teleostean character and development, is solid at first, hollowed out secondarily, and has no obvious connection with the yolk.

(2) No air-bladder has been detected at any stage.

(3) The pyloric valve arises by outpushings of the intestine. These blind cæcal outgrowths have the appearance of very short rudimentary pyloric cæca.

(4) Apart from these structures there are no pyloric cæca.

(5) The pancreas is an elongated compact gland arising from a dorsal and two ventral rudiments.

(6) The liver is elongated and unilobed.

(7) There is a typical thymus arising from clefts 2, 3, 4 and 5.

(8) A thyroid arises as a solid median derivative of the floor of the pharynx. It is elongated and bilobed anteriorly.

(9) The spleen develops early, is very conspicuous, and multilobed at first.

(C) Renal Organs.

(1) The pronephric chamber and tubule are formed from the nephrotome of the third trunk myotome.

(2) There is no communication at any time between splanchnocœle and nephrocœle of the pronephros.

(3) The archinephric duct is formed from the nephrotomes of the segments posterior to the third; the conversion of these nephrotomes into a duct takes place simultaneously, involving no backward growth of the archinephric duct.

(4) The pronephros is still present in the oldest larva examined.

(5) Mesonephric tubule-rudiments appear in Stage 29. They occur from about Segment 25 to Segment 43. Each arises as a rounded clump of darkly stained cells in the immediate neighbourhood of the archinephric duct. This rudiment is gradually moulded into a twisted tubule, one end of which becomes converted into a Malpighian capsule of the usual type, the other end acquiring an opening into the archinephric duct.

(6) There are no peritoneal funnels.

(7) Secondary mesonephric tubules arise in connection with the archinephric duct and with the primary mesonephric tubules. These are not fully differentiated in the oldest larva examined.

(8) The anterior much-coiled part of the archinephric duct, as well as the mesonephros, is surrounded by pseudo-lymphatic tissue.

(D) Vascular System.

(1) The development of the heart and vascular system agrees generally with that described for other Teleosteans.

(2) The free anterior part of the left posterior cardinal disappears, the large right posterior cardinal conveying the blood of the inter-renal vein to the heart.

(3) There is a close connection between the blood-vessels of the hinder ends of the kidney and liver recalling the posterior vena cava of Polypterus.

(4) The subintestinal vein, the front end of which is the vitelline vein of the earlier stages, persists in the adult as a hepatic vein. This hepatic vein joins up with the left anterior cardinal and left jugular to form the left ductus Cuvieri. The right ductus Cuvieri shows no special peculiarity.

(E) Nervous System.

(1) The brain is at first solid and is hollowed out secondarily.

(2) Three main divisions of the brain can be distinguished in Stage 21.

(3) There is no cranial flexure until Stage 24, and therefore no reason for assuming that the infundibulum is the morphologically anterior end of the brain.

(4) Sagittal sections through the brain at different stages show the usual Teleostean characters.

(5) The cerebellum is late in developing and goes on growing after metamorphosis.

(6) The optic lobes of the mature brain are relatively smaller than in the developing one. The mid-brain of the adult is the least conspicuous part.

(7) The mature brain is elongate, as also are the olfactory and optic nerves, the divisions well separated off.

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EXPLANATION OF PLATES 1—4.

Illustrating Miss Monica Taylor's paper on "The Development of Symbranchus."

PLATE 1.

Fig. 1.—A surface view of the youngest stage in the collection. The segmenting blastoderm looking like a white circular pad "let into" the yolk-mass occupies the centre of the flattened pole of the egg.

Fig. 2.—An egg of Stage 10. The blastoderm has a thickened rim, and at one point this rim becomes accentuated to form the embryonic rudiment.

Fig. 3.—An egg of Stage 11.

Fig. 4.—An egg of Stage 17.

Fig. 5.—An egg of Stage 21. *o. v.* Optic vesicles. *p. f.* Pectoral fins. *v. r.* Rudiment of visceral arches with their nerve supply.

Fig. 5A.—An egg of Stage 21, fixed in corrosive acetic. *b. r.* Branchial rudiment. *h. a.* Hyoid arch. *m. a.* Mandibular arch. *o. v.* Optic vesicles. *p. f.* Pectoral fins.

Fig. 6.—An egg of Stage 22.

Fig. 7.—An embryo of Stage 23. *c. s.* Cartilaginous skeleton of pectoral fin. *e.* Eye. *m.* Mesencephalon. *o.* Otocyst. *r. c.* Rhombencephalon. *r.* Rostrum.

Fig. 8.—Side view of an embryo of Stage 24. *e.* Eye. *n.* Nostril. *o.* Otocyst.

Fig. 9.—Larva of Stage 26. *a.* Anus. *c. r.* Cerebellum rudiment. *e.* Eye. *h.* Heart. *n.* Olfactory organ. *o. l.* Optic lobes. *op.* Operculum. *s. i. v.* Subintestinal vein.

Fig. 10.—A larva of Stage 30. *s. i. v.* Subintestinal vein. *y.* Yolk.

Fig. 11.—Sagittal section through an embryo of Stage 25. *i.* Inner and *o.* outer layer of ectoderm. *e.* Epiphysis. *m.* Mesencephalon. *pc.* Pericardium. *p.* Prosencephalon. *r.* Rhombencephalon. *v.* Ventricle.

PLATE 2.

Fig. 12.—Right pectoral fin and shoulder girdle of a larva of Stage 30.

Fig. 13.—A larva of Stage 32. *s. i. v.* Subintestinal vein. *y.* Yolk.

Fig. 14.—Ventral view of larva of Stage 33. *o. o.* Opercular opening.

Fig. 15.—A larva of Stage 33. *o. o.* Opercular opening.

Fig 16.—Stage 35.

PLATE 3.

Fig. 17.—Paraffin sagittal section through an egg, Stage 8. *s.* Space underlying blastoderm. *y. s.* Yolk syncytium.

Fig. 18.—A celloidin sagittal section, 25 μ thick, through an egg of Stage 10. *ec.* Ectoderm. *en.* Endoderm. *n.p.* Neural plate. *y.s.* Yolk syncytium. *y. s. n.* Yolk syncytium nuclei.

Fig. 19.—Transverse section through a larva of Stage 32. The operculum has grown backwards to the point of origin of the pectoral fins. *a. c. v.* Anterior cardinal vein. *d. a.* Dorsal aorta. *i. j.* Inferior jugular. *o.* Operculum. *s. a.* Subclavian artery. *s. g.* Shoulder girdle. *s. v.* Subclavian vein.

Fig. 20.—A horizontal section through a larva of Stage 25. *cœ.* Cœlom. *g. c.* Gill-chamber. *l.* Liver. *m.* Mesencephalon. *p.f.* Pectoral fin. *o.* Operculum. I, II, III, IV. Branchial arches 1–4.

Fig. 21.—A transverse section through a larva of Stage 26. *a. c.* Alimentary canal. *a. d.* Archinephric duct. *b. d.* Bile-duct. *cœ.* Cœlom. *d. a.* Dorsal aorta. *d. p.* Dorsal pancreas. *l.* Liver. *s.* Spleen. *y.* Yolk.

Fig. 22.—Sagittal section through a larva of Stage 34. The figure showing the brain is drawn from one section, that showing the lower jaw from another section, the distance between the two sections being 20 μ. *a. o.* Olfactory organ. *c.* Cerebellum. *c. pl.* Choroid plexus. *c. g.* Gill-cavity. *h. g.* Habenular ganglion. I. Infundibulum. *ol. l.* Olfactory lobes. *o. l.* Optic lobe. *thy.* Thyroid. *t.* Tongue. *v. t.* Velum transversum. II. Optic nerve.

PLATE 4.

Fig. 23.—Transverse section through a larva of Stage 29. *a. c. v.* Anterior cardinal vein. IV. *ef. bra.* Fourth efferent branchial. III, IV, *g. a.* Third and fourth branchial arch. *g. c.* Gill cavity. *p.* Pericardium. *p. o.* Pore-like opening into gill-chamber. *y. s.* Yolk-sac.

Fig. 24.—Transverse section through a larva of Stage 35. *d. a.* Dorsal aorta. *g. b.* Gall-bladder. *i. r. v.* Inter-renal vein. *l.* Liver. *plt.* Pseudo-lymphatic tissue surrounding kidney. *p. v.* Pyloric valve. *p. a.* Pancreas. *s.* Stomach (pylorus). *s. i. v.* Hepatic vein.

Fig. 25.—Sagittal section through an embryo of Stage 24. *a. c.* Alimentary canal. *d. a.* Dorsal aorta. *n.* Notochord. *s. c.* Spinal cord.

s.i.v. Sub-intestinal vein. *y.s.* Yolk syncytium. *y.s.n.* Yolk-syncytium nucleus.

Fig. 26.—Sagittal section through a larva of Stage 32. *a.* Atrium. *b.* Bulbus. *a.c.* Alimentary canal. *s.v.* Sinus venosus. *v.* Ventricle. *v.v.* Vitelline vein = sub-intestinal vein = portal vein. *y.s.* Yolk-syncytium.

Fig. 27.—Transverse section through a larva of Stage 33. *a.c.* Alimentary canal. *d.a.* Dorsal aorta. *g.* Glomerulus. *g.a.* Glomerular artery. *i.r.v.* Interrenal vein. *ia.* Intersegmental artery. *k.t.* Kidney tubules. *l.* Liver. *n.* Notochord. *y.* Yolk.

Fig. 28.—Brain dissected from a larva of Stage 34. A. Dorsal; B. Ventral view. *c.* Cerebellum. *l.l.* Lateral lobes of infundibulum. *m.o.* Medulla oblongata. *o.l.* Olfactory lobes. *t.* Telencephalon. *th.* Thalamencephalon. *t.o.* Tectum opticum.